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6. AUTHOR(S)
G. John Stanton
T. Kley Hughes

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)
The University of Texas Medical Branch
301 University Boulevard
Galveston, Texas 77551-1019

8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING/MONITORING AGENCY NAME(S) AND ADDRESS(ES)
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13. ABSTRACT (Maximum 200 words)

This report presents data that orally applied IFN can increase the levels of dehydroepiandrosterone (DHEA) sulfate in very young and old-age (20 months) mice and protect mice from Semliki Forest Virus infections. Large numbers of mice/group (42) were needed to show statistically significant increases in the splenic antibody plaque-forming response to sheep red blood cells. These increases were in the two to four fold range. Thus the biological significance needs to be proven. In other studies, DHEA and its more potent form, 5-androstene-3B, 17B-diol (AED) were shown to decrease ocular inflammatory responses and mortality, following ocular challenge with herpes simplex virus. Overall, the data suggest that oral IFN could help to increase DHEA-mediated responses during old age, and to modulate virus infections and immune responses. In addition, DHEA and AED could possibly be used to help decrease the inflammatory response during ocular infections.

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FINAL REPORT

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PRINCIPAL INVESTIGATOR: G. John Stanton and T. Kley Hughes

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OBJECTIVE: The overall objectives were to investigate whether Interferons given via the oral route could: modulate the production of the adrenal androgen, dehydroepiandrosterone sulfate (DHEAS), modulate immune responses, protect against Semliki Forest virus (SFV) and ocular herpes simplex virus (HSV) infections. In addition, the protective and immunomodulatory activities of DHEA and its more potent conversion product, 5-androstene-3B,17B-diol (AED) were investigated.

APPROACH: The approach was to give mice of various ages varying concentrations of mouse IFN α/β or recombinant human IFN AD, which cross-reacts in the mouse, *ad lib* in their drinking water, and sometimes ip. Depending on the experiments, we measured the quantities of DHEAS in serum by radio-immuno assay, the antibody plaque forming response to sheep red blood cells, and whether protection occurred against SFV and HSV infections. In other experiments DHEA or AED was injected sc and/or applied locally to the eye prior to and during HSV infection and the level of the inflammatory response and percent survival measured.

ACCOMPLISHMENTS: We found that overall, the DHEAS levels in plasma of ICR mice varied with age, in a manner similar to that of humans. Levels were generally low in weanling 4-week-old mice, highest in young adult 7-week-old mice and lowest in 20 month old-age mice. All levels differed by at least the < 0.025 level as determined by Student's t test. In the four-week-old groups, mice receiving 10 IU/ml had significantly higher levels of DHEAS in their plasma than did controls, $P = 0.010$. In contrast, levels in mice receiving 100 IU/ml were significantly lower than the control, $P = < 0.001$. Those receiving 300 or 1000 units had the same levels of DHEAS as the controls. Thus there is an indication that oral IFN may modulate DHEAS levels in mice of this age in a biphasic manner. In 7-week-old mice, orally applied IFN suppressed DHEAS levels below that of controls at all concentrations of IFN used ($P = < 0.001$) except 1000 IU/ml which did not differ significantly from the control level. Most interesting was the finding that low concentrations (10 and 30 IU/ml) of orally applied IFN significantly enhanced DHEAS levels in old-age mice, whereas 100 units increased the levels ($P = < 0.001$) and 300 IU/ml had no effect. The findings in the old age mice are suggestive, but should be considered preliminary until repeated. Interestingly, no differences in the survival times of mice in any of the treatment groups kept under our controlled laboratory conditions were noted.

We completed two studies in which groups of three C_3H mice were treated with oral IFN and immunized with 5 x 10^6 or 5 x 10^7 SRBC's, The results indicated that when a low level of SRBC's was used to immunize and spleens were assayed three days post immunization, there was a significant, 4 fold, increase in the number of measurable antibody producing cells (APC) per spleen in the group receiving 1000 IU IFN/ml (10.32 vs. 43.35, $p = 0.0034$ by Student's t test) than in the mock IFN treated controls. Groups receiving 1.0, 10, and 100 IU/ml had a 2 fold increase in APCs over controls ($p = 0.02$). The data suggest that when small antigen loads are used to immunize mice the kinetics of development of the immune response can be enhanced by higher concentrations of oral IFN. By the sixth day mice receiving 1 and 10 IU/ml IFN had significantly, 2.5 to 3 fold, more APCs than controls (69 vs. 214 and 164 respectively, $p = 0.006$ and 0.024 respectively). The APC response in mice receiving 1000 IU/ml, although highest on day three post immunization, was significantly higher, 2 fold, than the control and significantly lower, 1.9 fold, than mice receiving 1.0 IU IFN/ml ($p = 0.039$ and 0.008, respectively). These mice, however, did not differ significantly in their APC response from those receiving 10 and 100 IU/ml. Thus it appears that the overall response was biphasic; higher levels of IFN enhanced the early three day response but the magnitude of the response was better when lower levels of IFN were used. All levels of IFN used were better than mock IFN for enhancing the APC response.

In a similar study where a 10 fold higher concentration of SRBCs (5 x 10^7) was used to immunize mice, the early, three day, response was also significantly higher, 1.5 to 2 fold, when higher (100 and 1000 IU/ml) concentrations of oral IFN were used ($p = 0.001$ and 0.06 respectively). As might be expected no significant differences in the APC response was detected at five and six days post-immunization when this large immunizing dose was used. Overall these two studies indicate that oral IFN can modulate the immune response. However, although statistically significant increases in the PFC responses were observed in these studies, the increases were only in the two to four fold range, suggesting further studies are needed to determine the biological significance of the enhanced responses.

We have also shown that mice receiving oral IFN had increased survival rates following ip infection with SFV. Much larger groups of 6 week old ICR mice (20-22/group) were used in an attempt to ensure the statistical significance of the data obtained. We found that the combined data showed that mice treated orally with 10 and 3000 IU/ml were significantly protected when compared to controls ($p= 0.057$ and 0.018 by two-tailed Fisher's exact test, respectively). The survival rates were 7/42 for the groups receiving 10 units/ml, 9/41 for 3000/ml and 1/42 in the mock treated groups. This is the first time that significant protection has been observed in mice treated in this fashion when approximately 100% of the controls died. The responses observed were also biphasic as reported earlier.

In studies designed to determine the effects of DHEA and DHEA plus IFN on HSV infection, we found that 5µl of DHEA (0.5 mg/ml) applied ocularly before infection (1700 pfu/eye), or sc (1.0 g/kg) protected ICR mice from encephalitis and death ($p = 0.015$ and 0.06, respectively). DHEA + IFN at 200 and 2000 IU did not protect, thus no synergism was found at the levels of DHEA and IFN used. The same levels of IFN alone also did not protect. We observed similar findings in two other studies.

Also we found that DHEA (1g/kg) injected sc just before infecting could decrease or reverse the development of pathology in the eye, such as vascularity of the iris, cloudiness resulting from infiltration of cells and protein into the anterior chamber, and corneal and lid edema ($p>.05$). In contrast, DHEA (1g/kg) + IFN (10, 100, or 1000 IU/ml) applied locally were not as effective as DHEA alone. Together these findings indicate that DHEA alone works better than DHEA + IFN.

In other studies, we found that the protective effects of AED, the more potent form of DHEA, injected subcutaneously or administered to the eyes in drops did not protect ICR mice following ocular infection (17,000 pfu or 10 times the amount used in the previous experiments) with the virulent KOS strain of HSV. However, protection from encephalitis and death was observed in BALB/c mice treated subcutaneously (1.0 g/kg) and C_3H mice treated ocularly, 5 µl/eye, ($P = 0.08$ and 0.035 respectively by chi square analysis). Interestingly no protection occurred in C_3H mice treated subcutaneously or BALB/c mice treated ocularly. The data suggest that encephalitis and death from HSV infection originating in the eye can be significantly prevented following large challenges with HSV depending on the strain of mouse used.

We also found that the protective effects of AED correlated with the inflammatory response in the eye. Notably, the inflammatory responses in the eyes correlated directly with the mortality rates. The inflammatory response was severe in the ICR mice, marginal in the BALB/c and essentially absent in the C_3H mice. Further studies could elucidate whether the differences observed when higher concentrations of virus were used were due to variations in virus pathogenicity in the different strains of mice and/or variations in the effusiveness of AED.

CONCLUSIONS: We conclude from these studies that orally applied IFN has a statistically significant, but slight to moderate effect on the antibody plaque forming response and protective responses to SFV infection in mice. This fact and the fact that it is biphasic could make the proper dosages for humans very difficult to determine with out sufficient study. These studies also indicate that DHEA and AED can prevent development of inflammation and encephalitis in mice following ocular inoculation of HSV.

SIGNIFICANCE: Overall these studies indicate that orally applied IFN can modulate the DHEAS response. The responses appear to vary with the age of the mice being treated. The very young and the oldest mice had enhanced responses to treatment with low levels of IFN, while young adult mice generally had their responses suppressed at all levels of IFN except for those receiving 1000 IU/ml, in which the response was not significantly different from that of the controls. Other studies show for the first time that the antibody response can be significantly modulated by oral IFN administration and that the response is biphasic. In addition, these investigations show that oral IFN can significantly protect mice from a virus challenge that killed approximately 100% of the mock IFN treated mice. Local application of DHEA and AED to the eye have been shown to control the inflammatory response to HSV infection of the eyes and prevent distal spread to the brain. In addition these studies indicate for the first time that DHEA given sc can significantly prevent systemic effects, such as encephalitis and death, in a serious virus infection initiated at an epithelial surface, such as the eye. The development of the local pathology associated with this infection was also decreased or reversed.

PUBLICATIONS AND ABSTRACTS:

1. Stanton, G.J. and T.K. Hughes. (1995) Protection of mice from encephalitis by Dehydroepiandrosterone and 5-androstene-3B,17B-diol following ocular inoculation of Herpes simplex virus. In preparation

2. Rady, P.L., Cadet, P., Tyring, S.K., Baron, S., Stanton, G.J., and T.K. Hughes. Production of interferon-gamma messenger RNA by cells of non-immune origin. Submitted.